

## **Recent and rapid transmission of HIV among people who inject drugs in Scotland revealed through phylogenetic analysis**

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1 Recent and rapid transmission of HIV among  
2 people who inject drugs in Scotland revealed  
3 through phylogenetic analysis

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19 similar recent outbreaks in Greece, Romania, Ireland and the USA. Phylodynamic analysis  
20 demonstrates the infections are tightly linked genetically and the effective reproductive  
21 number remains above 1.

22

23

# 1 ABSTRACT

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Harm reduction has dramatically reduced HIV incidence among people who inject drugs (PWID). In Glasgow, Scotland, <10 infections/year have been diagnosed among PWID since the mid-90s. However, in 2015 a sharp rise in diagnoses was noted among PWID: many were subtype C with two identical drug resistant mutations and some displayed low avidity, suggesting the infections were linked and recent.

We collected Scottish *pol* sequences and identified closely related sequences from public databases. Genetic linkage was ascertained among 228 Scottish, 1820 UK and 524 global sequences. The outbreak cluster was extracted to estimate epidemic parameters.

All 104 outbreak sequences originated from Scotland and contained E138A and V179E. Mean genetic distance was <1% and mean time between transmissions was 6.7 months. The average number of onward transmissions consistently exceeded 1, indicating that spread was ongoing.

In contrast to other recent HIV outbreaks among PWID, harm reduction services were not clearly reduced in Scotland. Nonetheless, the high proportion of individuals with a history of homelessness (45%) suggests that services were inadequate for those in precarious living situations. The high prevalence of Hepatitis C (>90%) is indicative of sharing of injecting equipment.

Monitoring the epidemic phylogenetically in real-time may accelerate public health action.

## 2 INTRODUCTION

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People who inject drugs (PWID) are at risk of acquiring HIV from sharing injecting equipment and from high risk sexual activity while under the influence of drugs or in exchange for drugs[1]. There are 9-22 million PWID worldwide of whom 1-5 million have HIV [2].

Major outbreaks of HIV were identified among PWID in Scotland in the 1980s [3-5], along with other parts of northern [3, 6], and southern Europe[5]. A major outbreak in Edinburgh in 1983 associated with extensive needle-sharing [5] led to 50% of PWID becoming infected [3]. This epidemic was closely linked to similar ones in Dundee and Dublin [3], but few HIV cases were seen among PWID in Glasgow at the time[7]. Rapid introduction in the UK of needle exchange in 1986 followed by other harm reduction measures [8], dramatically decreased spread of HIV within this population. Since the mid-1990s HIV diagnoses among PWID in Glasgow have averaged 10 per year [9]. Similarly in the rest of Western Europe, incidence had declined in accordance with public health responses [10].

However, in 2011 there were reports of outbreaks of HIV among PWID in Greece [11], Romania [12], and Ireland [13]. Prior to this, HIV incidence among PWID in Greece and Ireland had been similar to the UK, around 0.1% [2, 14, 15]. In Greece, fewer than 20 infections per year were reported among PWID between 2001 and 2010, but in 2011 this surged to 256 cases accounting for ¼ of all new HIV diagnoses that year [16]. The epidemics in Greece [11] and Ireland [13] followed an economic crisis which led to increases in homelessness. The recession of 2008 resulted in funding cuts to opiate substitution therapy and needle exchange programs in Greece and Romania [17]. In parallel, the surge in injection of stimulant-based new psychoactive substances, which are typically injected more frequently than heroin thus increasing the risk of needle-sharing, contributed to the outbreaks in Romania [12] and Ireland [13].

In 2015 a significant rise in HIV diagnoses among PWID was noted in Glasgow. Data from Scotland's Needle Exchange Surveillance Initiative showed that HIV prevalence among PWID increased from 0.3% in 2011-12 to 1.9% in 2015-16 [18]. Routine sequencing to test for drug resistance revealed many were HIV subtype C, a subtype rarely observed among PWID in the UK [19, 20], suggesting a common source for the outbreak.

Reconstruction of the transmission network through contact tracing is difficult for HIV because of the time delay between infection and diagnosis, the low transmission rate, and the challenge of collecting information pertaining to sexual and drug-taking behaviours.

Phylogenetic analysis of viral sequences provides an alternative and independent route to reconstructing transmission networks [21]. As viral sequences are generated as a component of routine clinical care in the UK, we conducted a phylogenetic analysis to investigate whether PWID cases were related, when infections had been acquired, and whether the strain was spreading into the wider community and elsewhere in the UK.

Results informed the shape and intensity of the public health response.

## **3 METHODS**

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### **3.1 STUDY POPULATION**

Since 2005, the West of Scotland specialist virology centre has routinely carried out baseline sequencing of all new HIV diagnoses. The HIV-1 protease and reverse transcriptase regions (HXB2 positions 2253 to 3549) were amplified using primers described previously [22] with Expand reverse transcriptase and the Expand High Fidelity polymerase chain reaction (PCR) System (Roche) and the following programme: RT-PCR 42°C for 45 min; first round PCR (2 min at 94°C; 10 cycles of 15 sec at 94°C; 30 sec at 55 °C; 1 min 30 sec at 72°C; 25 cycles of 15 sec at 94°C; 30 sec at 55°C; 1 min 30 sec at 72°C + 5 sec/cycle; 10 min at 72°C ) and nested

PCR (2 min at 94°C; 10 cycles of 15 sec at 94°C; 30 sec at 55 °C; 1 min 30 sec at 72°C; 25 cycles of 15 sec at 94°C; 30 sec at 55°C; 1 min 30 sec at 72°C + 5 sec/cycle; 10 min at 72°C). Sanger sequencing was performed on the ABI3130xl using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Alignment and base-calling was performed using the online sequence analysis software RECall [23]. REGAv3 was used to subtype sequences and detect drug resistance mutations [24]. All subtype C sequences were extracted from the laboratory database for further analysis. At each stage (extraction through to PCR) and for each patient, negative controls were included in each assay to detect contamination. If evidence for contamination was observed, all patient samples in that run were re-extracted. For each weekly run a simple phylogenetic tree was constructed to detect contamination occurring at the sequencing stage. Any cases of sequence identity in the same batch were repeated from the extraction stage.

Avidity testing was used to classify infections as recent or older than four months. The assay is a modification of the Genscreen HIV1/2 Version 2 (Bio-Rad) [25] and testing has been performed on HIV diagnoses since 2012. Clinical and epidemiological information was obtained through the National Health Service clinical portal, a virtual electronic patient record that contains clinical notes on interactions with tertiary services and test results. Data retrieved included hepatitis C status, viral load, date of last HIV negative test, sex, risk group, age, nationality, and history of drug use, incarceration and homelessness.

## **3.2 BACKGROUND SEQUENCES**

The UK HIV Drug Resistance Database (UKRDB) receives *pol* sequences obtained for routine clinical surveillance and submitted by participating laboratories. Resistance data are linked to demographic and clinical patient data held in the UK Collaborative HIV Cohort study (UK CHIC) database [26] and the national HIV/AIDS Reporting System (HARS) database held at Public Health England[27]. After linking sequences to epidemiological data, the data were

anonymised. In the 2014 release of the database (sequences up to mid-2013), sequences were available for around 60% of the infected population and >80% of patients diagnosed since 2005. Of 63,163 sequences in the UKRDB, 15,864 sequences (25.1%) were classified as subtype C by REGAv3 [24]. Epidemiological data contributed by Public Health England and Health Protection Scotland included year of birth, gender and self-reported most likely route of infection (PWID, heterosexual sex, men who have sex with men (MSM), mother to child, blood product, or unknown).

The Los Alamos National Laboratory (LANL) HIV database compiles all published HIV sequences, including 11,658 non-UK subtype C *pol* sequences (accessed 8<sup>th</sup> August 2014). We used Geneious to BLAST Scottish sequences against UKRDB and LANL sequences, selecting the ten closest matches for each individual Scottish sequence for analysis [28]. As the same sequence is hit multiple times, this procedure limits the size of the final alignments. Final alignments comprised 228 sequences from Scotland, 1820 from the UKRDB and 524 from LANL (2572 in total).

### **3.3 GENETIC LINKAGE AND PHYLODYNAMIC ANALYSIS**

Sequences were stripped of 44 sites associated with drug resistance based on the 2013 International AIDS Society list [29]. We reconstructed genetic clusters by calculating genetic distances between pairs of sequences under a TN93 substitution model. Each sequence was represented in the network by a node and nodes were linked if their pairwise distance was below a certain genetic distance threshold. At thresholds 1-2.5%, the same PWID outbreak cluster stood out (n=104, see Results), with all sequences from Scotland. We selected the tightest threshold because 1% is consistent with recent and rapid transmission [30]. 10% of outbreak sequences were submitted to Genbank (Accession numbers MG76186:MG761826). Sequences from the outbreak were further analysed using the Bayesian birth-death skyline model in BEAST2 [31, 32]. The birth-death skyline is well suited

to analysing outbreaks, because unlike coalescent models, it does not assume low sample fraction within an infinite population size; instead, sample fraction is explicitly parameterized. Furthermore, the birth-death skyline estimates the effective reproductive number  $R_e$ , the average number of infections originating from each individual, directly yielding epidemiologically-relevant results. Substitution models (TN93, GTR+G+I) and clock models (strict, uncorrelated lognormal) were compared and a GTR+G+I model with an uncorrelated lognormal clock was selected based on its Bayes factor. Chain samples were run for 500,000,000 generations in triplicate. Prior distributions for  $R_e$  and the rate of becoming non-infectious were extracted from a previous analysis of the UK epidemic[31], and priors for the origin of the tree and the sampling proportion were based on our knowledge of the UK epidemic. The origin of the tree was set with a maximum value of 30 years and the sampling proportion was set as 0 until 2005 (the date of the first outbreak sequence) then with a normal distribution with mean=0.65 and sd=0.05. Because sampling fraction,  $R_e$  and time to becoming non-infectious are correlated in the birth-death skyline model, at least one must be set with a narrow prior [31].

## 4 RESULTS

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### 4.1 THE DRUG-RESISTANT SUBTYPE C OUTBREAK HAS NOT BEEN OBSERVED OUTSIDE SCOTLAND

All Scottish subtype C sequences were included in the phylogenetic analysis ( $n=228$ ), alongside 1820 sequences from the UKRDB and 524 from LANL (2572 in total). Of 2572 sequences, 501 (19.5%) linked to at least one other in the network using a genetic distance cut-off of 1%.



Within the network, a tight cluster of 104 sequences stood out (Figure 1). All sequences within the cluster were less than 1% genetic distance from at least one other sequence in the cluster. Mean genetic distance was <1% with 7 patients with *pol* sequences exactly identical to another, 2 pairs and 1 triad. All sequences originated from Scotland and contained E138A and V179E. Thus we have not yet observed evidence of spread of this strain outside Scotland. E138A is a common polymorphic accessory mutation weakly selected in patients receiving etravirine and rilpivirine that reduces susceptibility to these two antiretrovirals by around two fold. V179E is a non-polymorphic mutation weakly selected by nevirapine and efavirenz and associated with resistance to nevirapine, efavirenz and etravirine. In the UKRDB, which includes sequences sampled in Scotland until mid-2013, E138A is found in 1648/15,864 of subtype C sequences (10.39%) and V179E is found in 50 (0.32%). Only 41 sequences in the UKRDB contained both mutations (0.25%), of which 26 were from the present outbreak. Among the remaining fifteen, twelve sequences comprising both mutations were not closely related to the outbreak and were not included in the phylogenetic analysis; and three were included in the analysis but did not link to the outbreak. Between 2014 and mid-2016, 5 non-outbreak HIV diagnoses were made among PWID in Scotland.

## **4.2 SPREAD OF HIV AMONG SCOTTISH PWID HAS BEEN RECENT AND RAPID**

Sequences from the outbreak cluster (n=104) were selected for analysis using the birth-death skyline models in BEAST2 to estimate growth through time and to better infer the origin of the cluster. Runs converged within 5,000,000 generations with ESS values above 200.

The common ancestor to the cluster was dated as mid-2003 (2003.4; 95%HPD: 2001.8-2005.0), while the oldest outbreak sequence was from a female PWID diagnosed in 2005. Five patients were diagnosed in 2008-2009 (4.8%), 27 were diagnosed between 2010 and

2013 (26.0%) and 71 patients were diagnosed after 2014 (68.3%). All were diagnosed in Scotland and all those with a risk group reported were PWID.

The birth death skyline infers the effective reproductive number  $R_e$  (the average number of transmissions for each individual). Importantly,  $R_e$  has remained above 1 throughout the course of the outbreak (Figure 2), implying that the number of cases would be expected to continue to rise. Mean  $R_e$  was estimated as 1.5 (95%HPD 0.1-3.9) over the course of the outbreak, rising to 1.8 (HPD 1.1-2.6) during the last 2 years. At its highest point, in 2009,  $R_e$  exceeded 2. Sample fraction was estimated as 0.66 (HPD 0.46 -0.81).

The distance between internal nodes in the tree approximates the upper bound on time between transmission events [33]. Based on the time-resolved trees, the average transmission interval was estimated as 6.7 months for the outbreak as a whole (Supplementary Information). Looking at the phylogeny in more detail, it divided into three subclusters: 1a, 1b, and 2, originating in peak2 and 2010, respectively (Figure 3). Subclusters 1a and 1b had a higher density of recent transmission events, but there was no difference in transmission intervals between subclusters based on an analysis of 1,000 trees from the posterior distribution (Supplementary Information), indicating that while subclusters 1a and 1b are most active at present, the transmission dynamics within all three subclusters have been similar. The origin of subclusters 1a and 1b correlated with an increase in  $R_e$  (Figure 2).

### **4.3 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF OUTBREAK MEMBERS**

Among the 104 individuals in the outbreak diagnosed by mid-2016, 102 (98.1%) reported injecting drugs. Mean age was 38.4 (SD=6.5), 63/103 (61.2%) were men and 40 (38.8%) were women, 99/100 were white British (1 mixed race), 38/96 (39.6%) had a recorded history of incarceration and 41/92 (44.6%) reported having ever been homeless. 96/98 (98.0%) were confirmed to be HCV antibody positive, with 6 not tested, suggesting wide-spread sharing of

injecting equipment. 68/96 (70.8%) had ongoing HCV infection with a positive HCV antigen or PCR result.

HIV avidity was tested on 87/104 (83.7%) patients and 49/104 has a previous negative test result. 24/87 (27.6%) had low avidity results indicating that infection had occurred within the last four months. Five additional patients had a date of last negative HIV test less than a year previous to their diagnosis. Three patients had antibody levels too low for avidity testing indicating acute seroconversion, confirmed with negative Western Blot and BiSpot results. Therefore, in total at least 32/87 (36.8%) had HIV for less than a year at diagnosis, consistent with the short terminal branch lengths in the phylogenetic tree (Figure 3).

## 5 DISCUSSION

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This recent outbreak in Scotland is the latest in a series of rapid transmissions of HIV among PWID; in Greece [11], Romania [12], Ireland [13] and Indiana, USA [34]. Prior to these outbreaks, HIV incidence among PWID in these regions had been fairly static since the epidemics of the 1980s. From 2001 to 2014 there were 10-20 new cases per year in Scotland with 5-10 new cases around Glasgow [35]. The Scottish outbreak now comprises over one hundred linked cases.

Phylogenetic analysis demonstrated how rapidly the virus was transmitted, with average transmission intervals around 6 months, similar to MSM [33] and shorter than heterosexuals [36] in the UK. In contrast to the more commonly presented  $R_0$ , which represents the number of onward transmissions in a fully susceptible population,  $R_e$  is the number of secondary infections for the current frequency of susceptibles [37]. The number of secondary infections has averaged 1.5 since the outbreak originated around 2003, reaching 2 at its peak in 2009. In contrast, HIV diagnoses among PWID did not reach a peak until 2015 in Scotland remained around 20 per year between 2008 and 2010, not reaching a peak until

235 2015[38]. UK estimates of  $R_e$  for heterosexuals and MSM are below 1, and just above 1,  
236 respectively [39]. Previous estimates of  $R_e$  among PWID have ranged as high as 21.7 in  
237 Lithuania [40]. For the UK, estimates of  $R_e$  do not exist for PWID, but  $R_e$  was consistently  
238 above 1 for this outbreak, indicating that spread was ongoing in 2016. This number is  
239 specific to this outbreak and should not be extrapolated to reflect HIV transmission among  
240 PWID in the UK in general. The outbreak subdivided into subclusters, indicating independent  
241 concurrent transmission chains. All three transmission chains showed evidence of recent  
242 transmission events, and had equally short transmission intervals.

243 Genetic distance within the outbreak was extremely low, with multiple sets of identical  
244 partial *pol* sequences, a phenomenon observed in cases of rapid transmission [41]. While in  
245 part due to the short region of the virus sequenced, such low divergence demonstrates how  
246 rapidly the virus spread in this group. The potential for multiple partners during acute  
247 infection leads to low genetic diversity within PWID transmission networks. The recent  
248 outbreak among Greek PWID similarly displayed low diversity and high clustering[11],  
249 reminiscent of the spread of near identical subtype A variants throughout Eastern European  
250 and Russian PWID in the 1990s [42]. Full genome sequencing, currently being undertaken,  
251 may disentangle the sequence of transmissions within the outbreak.

252 The outbreak was in part detected because of two resistance mutations, E138A and V179E,  
253 repeatedly identified in subtype C viruses, which had not previously associated with PWID  
254 infection in the UK. This prompted a search through the UKRDB for the mutations and for  
255 related sequences. At present, despite the UKRDB collecting sequences from all HIV  
256 resistance tests, sequences are used for research purposes and not as a systematic  
257 surveillance tool. Genetic analysis confirmed the strain was unique to Scotland and is not  
258 transmitting elsewhere in the UK. In the UK it is rare to find large clusters from a single  
259 region [43], and this is now the largest cluster of HIV among PWID seen in the UK since the

1980s. However, we did not have samples from the rest of the UK as recent as those from Scotland. The most recent Scottish sequence was sampled in 2016, whereas the most recent UKRDB sequence was sampled in 2013. It is possible that the outbreak has spread outside Scotland but that we have not captured it.

No published PWID outbreaks have reported transmission of resistance mutations, although preliminary results from Saskatchewan, Canada, demonstrated the G190 mutation disproportionately affected Aboriginal PWID[44]. Earlier studies found a higher prevalence of resistance mutations among PWID than among those infected sexually[45]. Suboptimal treatment adherence among this group may provide an explanation for this phenomenon[46]. Another possibility is that blood to blood transmission could enable transmission of lower fitness viruses unable to establish infection through sexual transmission[47].

Despite access to injecting equipment, HIV still poses a significant risk to PWID. The identification of a unique strain facilitated its detection in Scotland during this outbreak, but real-time monitoring may help accelerate public health action. British Columbia recently deployed a real-time phylogeny response, where monthly reports were generated detailing cluster growth[48]. This analysis revealed a highly active cluster that expanded by eleven cases in three months. Members of the cluster were contacted to ensure linkage to care and partner notification and subsequently no further cases linked to those members were diagnosed. In the case of the Scottish outbreak, real-time phylogenetic monitoring could have brought the cluster to attention sooner. At present all UKRDB analyses are conducted with anonymised data, while Poon *et al* identified subjects to reach out to them. Use of non-anonymised HIV data for phylogenetic analyses is avoided in some jurisdictions because of the criminalisation of HIV transmission. An anonymised version of Poon's system can also be imagined, in which the background of sequences for comparison is anonymous but data are

285 available for the patient being seen at that moment [49]. If the patient's sequence were to  
286 cluster with two or more recent sequences, that patient could be selected for early initiation  
287 of treatment and pre-exposure prophylaxis could be offered to their partners. The  
288 advantage of Poon's method is that all members of the cluster can be retrospectively  
289 contacted whereas under the anonymised system, only patients diagnosed after the first  
290 few in a cluster would be identified. Overall, advances such as avidity testing and real-time  
291 phylogenetic analysis can be used to improve our understanding of outbreaks to better  
292 target public health responses.

293 Many PWID involved in the outbreak had experienced homelessness. Scotland's Needle  
294 Exchange Surveillance Initiative emphasised this point: almost 90% (20/23) of PWID from  
295 Glasgow who tested positive for HIV in 2015-16 had a history of homelessness, three-  
296 quarters of whom had been homeless within the last 6-months [18]. The situation in  
297 Scotland differs from that in other PWID outbreaks, however, because harm reduction  
298 services (Injecting Equipment Provision, Opiate Substitution Therapy) were available in  
299 Scotland post-recession. Indeed, Glasgow operates one of the most active Injecting  
300 Equipment Provision service in Europe, distributing over one million syringes per year[18]. In  
301 contrast, in Indiana, neither needle exchange nor HIV testing were available at the time of  
302 the outbreak[34]. Nonetheless, the association observed with homelessness suggests that  
303 harm reduction services available in Glasgow may have been difficult to access for those in  
304 precarious living situations, often with chaotic lifestyles.

305 This outbreak may have been due to a change in circumstances, but it may result from the  
306 unfortunate introduction of HIV into a group of connected but previously uninfected PWID,  
307 such as was the case in Sweden in 2006 [50] and in Indiana in 2015 [34]. The high prevalence  
308 of hepatitis C among PWID in this outbreak (>90%) is indicative of widespread injecting  
309 equipment sharing. In contrast, in Romania and Greece, multiple strains and networks were

uncovered [11, 12]; these outbreaks resulted from the reduced availability of harm reduction services. The Scottish outbreak is being managed through education of the population at risk and service providers, improved addiction services, increasing provision of needle exchange (e.g. greater evening availability), improving accessibility of HIV testing, and outreach services to support early treatment and retention. Further research is needed to demonstrate whether homelessness, or other behavioural factors, played a role in the outbreak.

# 6 FIGURES

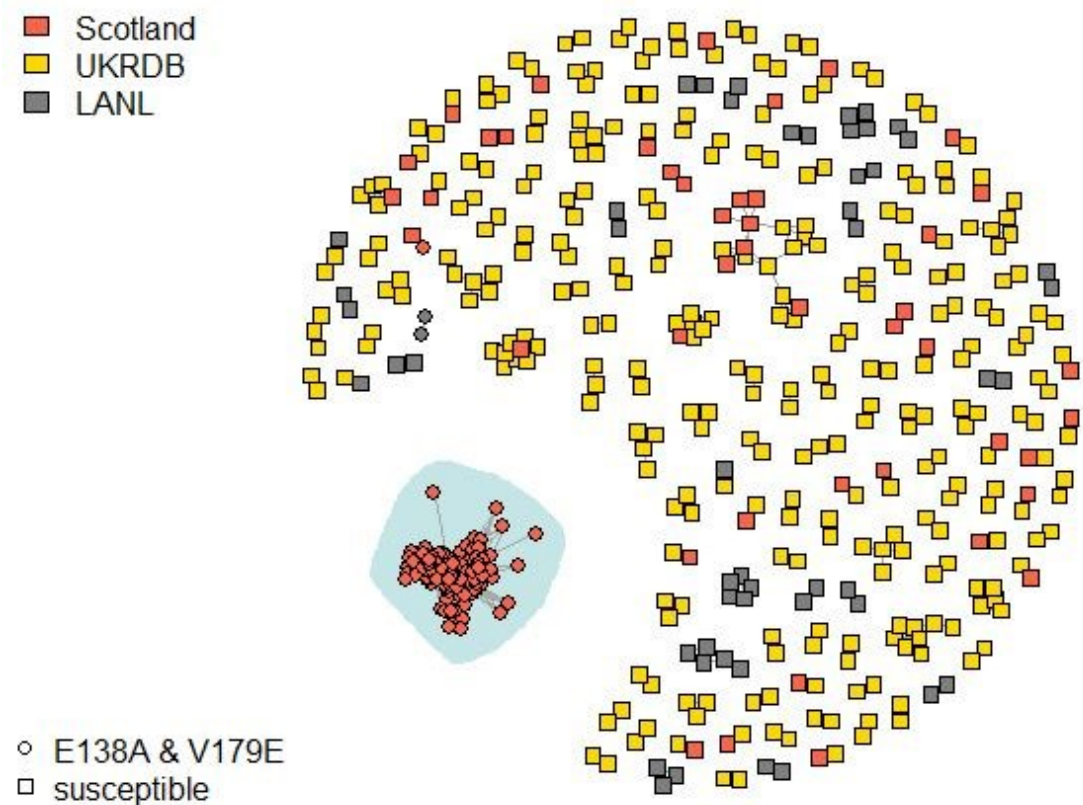
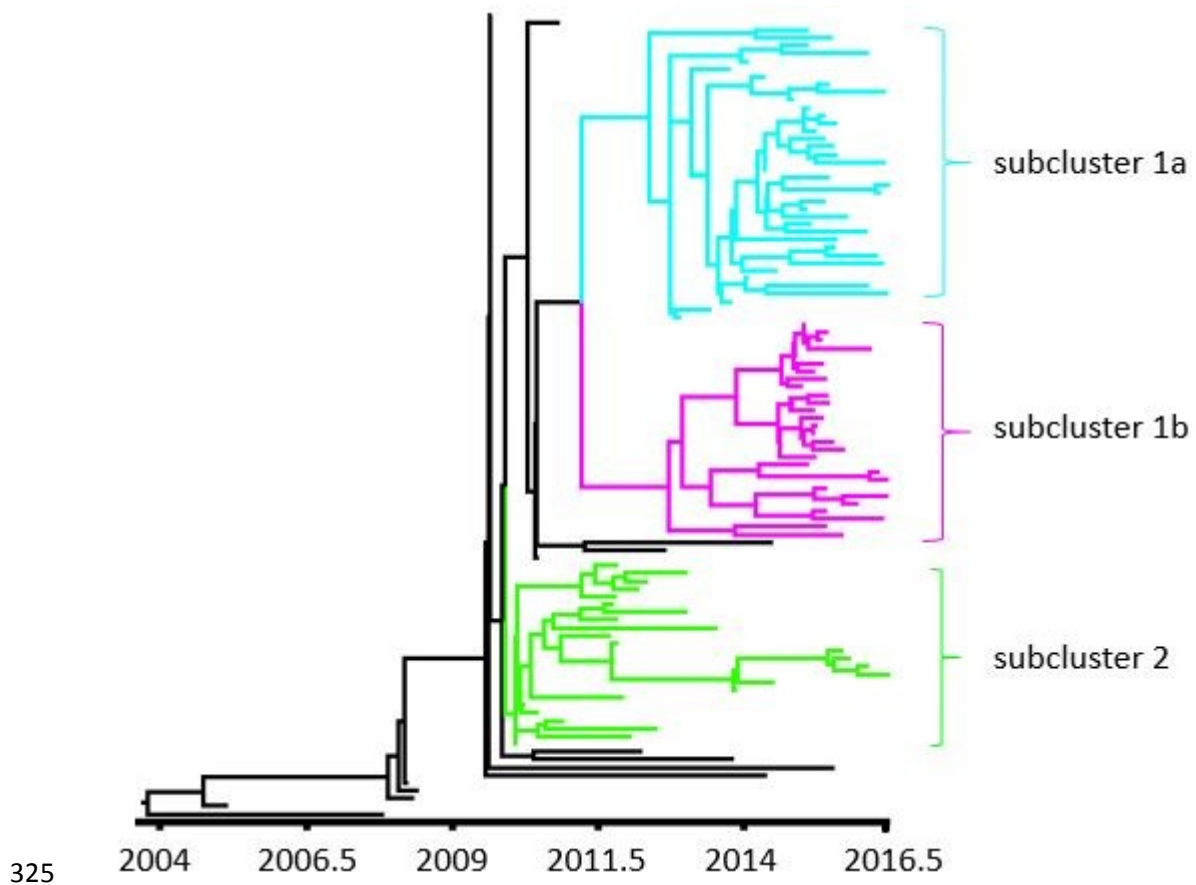


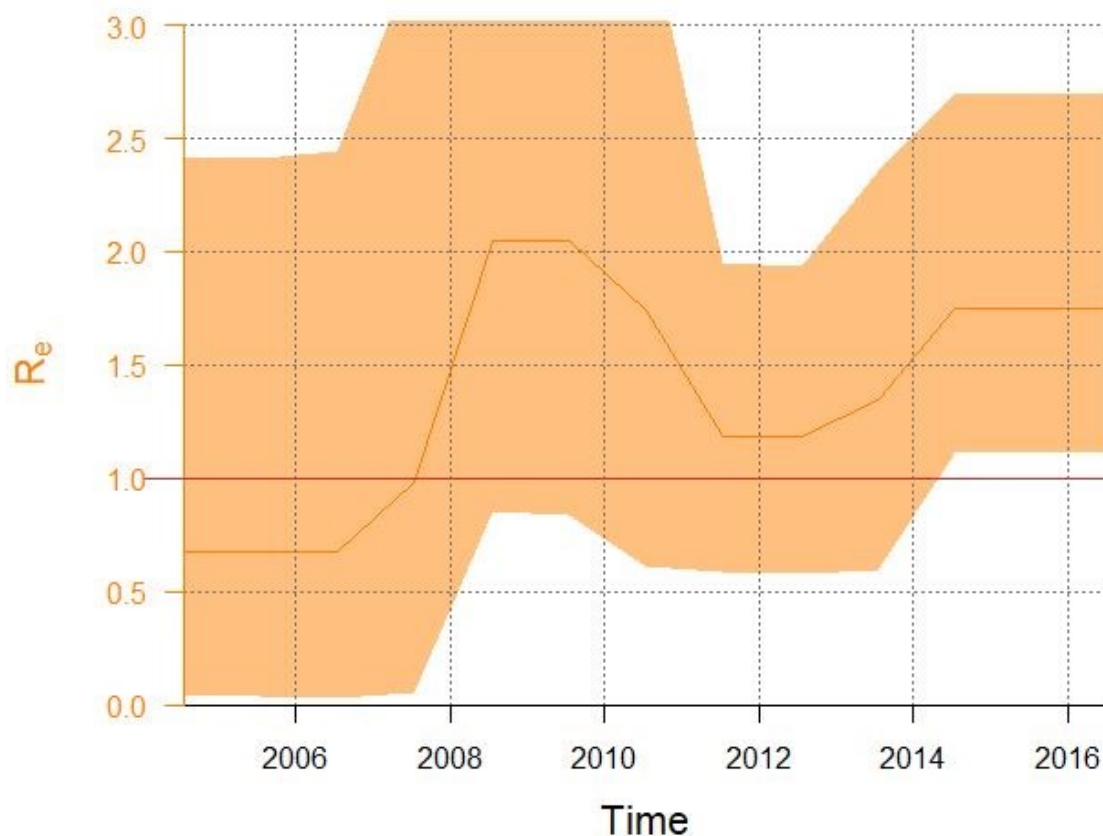
Figure 1: Genetic distance network of relatedness at 1%. Of 2572 sequences analysed, only those linked to at least one other sequence at 1% are shown (501 in total). Sequences are coloured by origin: Scotland, the UK HIV Drug Resistance Database (UKRDB) or Los Alamos National Database (LANL). Node shapes are determined by drug susceptibility of viruses.

323 One large cluster, highlighted and circled, stood out due to its size (104 sequences), its  
324 concentration of drug-resistant sequences and its Scottish origin.



326 Figure 2. Reproductive number  $R_e$  inferred from the birth death skyline plot. The line across  
327 the plot marks  $R_e=1$ , the threshold above which an infection will continue to spread.





329

330 Figure 3: Time-resolved phylogeny for the outbreak cluster comprising 104 sequences from  
 331 Scotland with drug resistant mutations E138A and V179E. The outbreak subdivided into  
 332 three subclusters.

## 333 7 FOOTNOTES

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### 334 7.1 CONFLICT OF INTEREST

335 MRC is currently supported by a grant from Gilead to the University of California, San Diego  
 336 for work on Hepatitis C. This funding was received after the present work was completed  
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### 338 7.2 MEETINGS WHERE THE WORKS HAS BEEN PRESENTED

339 Conference on Retroviruses and Opportunistic Infections, Seattle, USA, 2017

340 HIV Dynamics and Evolution, Isle of Skye, Scotland, 2017

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- 360 1. United Nations Office on Drugs and Crime. 2005 World Drug Report  
361 Volume 1: Analysis. Geneva, **2005**.
- 362 2. Des Jarlais DC, Kerr T, Carrieri P, Feelemyer J, Arasteh K. HIV infection  
363 among persons who inject drugs: ending old epidemics and addressing new  
364 outbreaks. *AIDS* **2016**; 30:815-26.
- 365 3. Leigh Brown AJ, Lobidel D, Wade CM, et al. The molecular epidemiology of  
366 human immunodeficiency virus type 1 in six cities in Britain and Ireland.  
367 *Virology* **1997**; 235:166-77.
- 368 4. Holmes EC, Zhang LQ, Robertson P, et al. The molecular epidemiology of  
369 human immunodeficiency virus type 1 in Edinburgh. *J Infect Dis* **1995**; 171:45-  
370 53.
- 371 5. Robertson JR, Bucknall AB, Welsby PD, et al. Epidemic of AIDS related  
372 virus (HTLV-III/LAV) infection among intravenous drug abusers. *Br Med J*  
373 (Clin Res Ed) **1986**; 292:527-9.
- 374 6. Op de Coul EL, Prins M, Cornelissen M, et al. Using phylogenetic analysis  
375 to trace HIV-1 migration among western European injecting drug users  
376 seroconverting from 1984 to 1997. *AIDS* **2001**; 15:257-66.
- 377 7. Davies AG, Cormack RM, Richardson AM. Estimation of injecting drug  
378 users in the City of Edinburgh, Scotland, and number infected with human  
379 immunodeficiency virus. *Int J Epidemiol* **1999**; 28:117-21.
- 380 8. Stimson GV. AIDS and injecting drug use in the United Kingdom, 1987-  
381 1993: the policy response and the prevention of the epidemic. *Soc Sci Med*  
382 **1995**; 41:699-716.
- 383 9. Public Health England, Health Protection Scotland, Public Health Wales,  
384 Public Health Agency Northern Ireland. Shooting Up: Infections among people  
385 who inject drugs in the UK, 2016. London, **2017**.
- 386 10. Hamers FF, Batter V, Downs AM, Alix J, Cazein F, Brunet JB. The HIV  
387 epidemic associated with injecting drug use in Europe: geographic and time  
388 trends. *AIDS* **1997**; 11:1365-74.
- 389 11. Sypsa V, Paraskevis D, Malliori M, et al. Homelessness and Other Risk  
390 Factors for HIV Infection in the Current Outbreak Among Injection Drug Users  
391 in Athens, Greece. *Am J Public Health* **2015**; 105:196-204.
- 392 12. Botescu A, Abagiu A, Mardarescu M, Ursan M. HIV/AIDS among injecting  
393 drug users in Romania: Report of a recent outbreak and initial response  
394 policies, **2016**.
- 395 13. Giese C, Igoe D, Gibbons Z, et al. Injection of new psychoactive  
396 substance snow blow associated with recently acquired HIV infections among  
397 homeless people who inject drugs in Dublin, Ireland, 2015. *Euro Surveill*  
398 **2015**; 20.
- 399 14. Yin Z, Brown AE, Hughes G, Nardone A, Gill ON, Delpech V. HIV in the  
400 United Kingdom: 2014 Report. Public Health England, London, **2014**.
- 401 15. Public Health England, Health Protection Scotland, Public Health Wales,  
402 Public Health Agency Northern Ireland. Shooting Up: Infections among people  
403 who inject drugs in the UK, 2014. London, **2015**.
- 404 16. Fotiou A, Micha K, Paraskevis D, Terzidou M, Malliori M, Hatzakis A. An  
405 updated report for the EMCDDA on the recent outbreak of HIV infections

among drug injectors in Greece. In: Addiction EMCfDaD, ed. Athens, Greece, **2012**.

17. Hedrich D, Kalamara E, Sfetcu O, et al. Human immunodeficiency virus among people who inject drugs: is risk increasing in Europe? *Euro Surveill* **2013**; 18:20648.

18. University of the West of Scotland, Health Protection Scotland, Glasgow Caledonian University, West of Scotland Specialist Virology Centre. The Needle Exchange Surveillance Initiative (NESI): Prevalence of HCV and injecting risk behaviours among people who inject drugs (PWID) attending injecting equipment provision services (IEPs) in Scotland, 2008/2009 - 2015/2016. In: Health Protection Scotland, ed. **Glasgow, UK, 2016**.

19. The UK Collaborative Group on HIV Drug Resistance. The increasing genetic diversity of HIV-1 in the UK, 2002-2010. *AIDS* **2014**; 28:773-80.

20. Ragonnet-Cronin M, Lycett SJ, Hodcroft EB, et al. Transmission of Non-B HIV Subtypes in the United Kingdom Is Increasingly Driven by Large Non-Heterosexual Transmission Clusters. *J Infect Dis* **2016**; 213:1410-8.

21. Grenfell BT, Pybus OG, Gog JR, et al. Unifying the epidemiological and evolutionary dynamics of pathogens. *Science* **2004**; 303:327-32.

22. Alexander CS, Dong W, Chan K, et al. HIV protease and reverse transcriptase variation and therapy outcome in antiretroviral-naïve individuals from a large North American cohort. *AIDS* **2001**; 15:601-7.

23. Woods CK, Brumme CJ, Liu TF, et al. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *J Clin Microbiol* **2012**; 50:1936-42.

24. Pineda-Pena AC, Faria NR, Imbrechts S, et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. *Infect Genet Evol* **2013**; 19:337-48.

25. Shepherd SJ, McAllister G, Kean J, et al. Development of an avidity assay for detection of recent HIV infections. *J Virol Methods* **2015**; 217:42-9.

26. UK Collaborative HIV Cohort Steering Committee. The creation of a large UK-based multicentre cohort of HIV-infected individuals: The UK Collaborative HIV Cohort (UK CHIC) Study. *HIV Med* **2004**; 5:115-24.

27. Public Health England. National HIV/AIDS Reporting System (HARS), **2008**.

28. Kearse M, Moir R, Wilson A, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**; 28:1647-9.

29. Johnson VA, Calvez V, Gunthard HF, et al. Update of the drug resistance mutations in HIV-1: March 2013. *Top Antivir Med* **2013**; 21:6-14.

30. Smith DM, May SJ, Twesten S, et al. A public health model for the molecular surveillance of HIV transmission in San Diego, California. *AIDS* **2009**; 23:225-32.

31. Stadler T, Kuhnert D, Bonhoeffer S, Drummond AJ. Birth-death skyline plot reveals temporal changes of epidemic spread in HIV and hepatitis C virus (HCV). *Proc Natl Acad Sci U S A* **2013**; 110:228-33.

32. Bouckaert R, Heled J, Kuhnert D, et al. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* **2014**; 10:e1003537.

33. Lewis F, Hughes GJ, Rambaut A, Pozniak A, Leigh Brown AJ. Episodic sexual transmission of HIV revealed by molecular phylodynamics. *PLoS Med* **2008**; 5:e50.
34. Conrad C, Bradley HM, Broz D, et al. Community Outbreak of HIV Infection Linked to Injection Drug Use of Oxycodone--Indiana, 2015. *MMWR Morb Mortal Wkly Rep* **2015**; 64:443-4.
35. Health Protection Scotland. HIV-infected persons, Scotland: Number of cases reported by NHS Board, transmission category, and year of report; to 31 December 2012: Health Protection Scotland, **2013**.
36. Hughes GJ, Fearnhill E, Dunn D, Lycett SJ, Rambaut A, Leigh Brown AJ. Molecular phylodynamics of the heterosexual HIV epidemic in the United Kingdom. *PLoS Pathog* **2009**; 5:e1000590.
37. Amundsen EJ, Stigum H, Rottingen JA, Aalen OO. Definition and estimation of an actual reproduction number describing past infectious disease transmission: application to HIV epidemics among homosexual men in Denmark, Norway and Sweden. *Epidemiol Infect* **2004**; 132:1139-49.
38. Public Health England, Health Protection Scotland, Public Health Wales, Public Health Agency Northern Ireland. Shooting Up: Infections among people who inject drugs in the UK, 2016. Accompanying data tables. London, **2017**.
39. White PJ, Ward H, Garnett GP. Is HIV out of control in the UK? An example of analysing patterns of HIV spreading using incidence-to-prevalence ratios. *AIDS* **2006**; 20:1898-901.
40. Stadler T, Kouyos R, von Wyl V, et al. Estimating the basic reproductive number from viral sequence data. *Mol Biol Evol* **2012**; 29:347-57.
41. Brooks JT, Robbins KE, Youngpairoj AS, et al. Molecular analysis of HIV strains from a cluster of worker infections in the adult film industry, Los Angeles 2004. *AIDS* **2006**; 20:923-8.
42. Bobkov A, Kazennova E, Khanina T, et al. An HIV type 1 subtype A strain of low genetic diversity continues to spread among injecting drug users in Russia: study of the new local outbreaks in Moscow and Irkutsk. *AIDS Res Hum Retroviruses* **2001**; 17:257-61.
43. Ragonnet-Cronin M, Hodcroft E, Hue S, et al. Automated analysis of phylogenetic clusters. *BMC Bioinformatics* **2013**; 14:317.
44. Wong A, Kambo J, Harrigan PR, Poon AF, Joy J. Large NNRTI-resistant transmission cluster in injection drug users in Saskatchewan. In: Conference on Retroviruses and Opportunistic Infections. **2015**.
45. Pham QD, Do NT, Le YN, et al. Pretreatment HIV-1 drug resistance to first-line drugs: results from a baseline assessment of a large cohort initiating ART in Vietnam, 2009-10. *J Antimicrob Chemother* **2015**; 70:941-7.
46. Jordan MR, La H, Nguyen HD, et al. Correlates of HIV-1 viral suppression in a cohort of HIV-positive drug users receiving antiretroviral therapy in Hanoi, Vietnam. *Int J STD AIDS* **2009**; 20:418-22.
47. Leigh Brown AJ, Frost SD, Mathews WC, et al. Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population. *J Infect Dis* **2003**; 187:683-6.
48. Poon AF, Gustafson R, Daly P, et al. Near real-time monitoring of HIV transmission hotspots from routine HIV genotyping: an implementation case study. *Lancet HIV* **2016**; 3:e231-e8.

- 503 49. Brooks JI, Sandstrom PA. The power and pitfalls of HIV phylogenetics in  
504 public health. *Can J Public Health* **2013**; 104:e348-e50.
- 505 50. Skar H, Axelsson M, Berggren I, et al. Dynamics of two separate but  
506 linked HIV-1 CRF01\_AE outbreaks among injection drug users in Stockholm,  
507 Sweden, and Helsinki, Finland. *J Virol* **2011**; 85:510-8.

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